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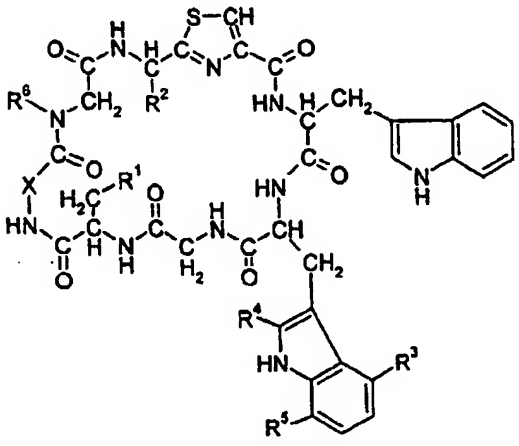
(54) Abstract Title
Pharmaceutically Active Macrocycles

(57) Compounds of the formula:



(I)

wherein
R^1, R^2 and R^3 independently are hydrogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;
R^4 is hydrogen, halogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;
R^5 is hydrogen or halogen;
R^6 is hydrogen or C_1-C_4alkyl; and
X is $C{=}CH_2$ or CHR^6 wherein R^6 is C_1-C_4alkyl which is unsubstituted or substituted by -S-C_1-C_4alkyl,
are useful against autoimmune disorders or diseases.

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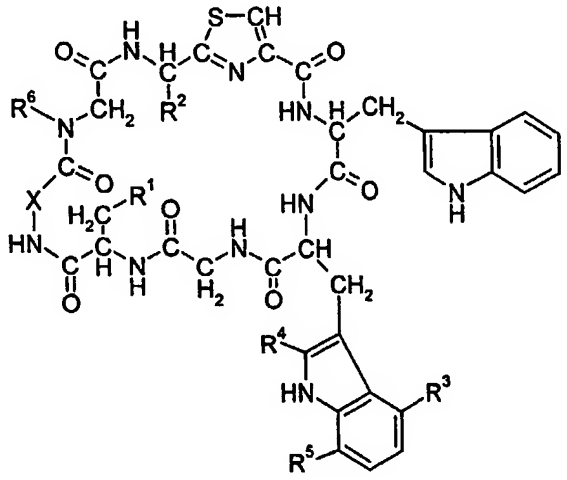
Organic Compounds

The invention is directed to novel macrocycles, their use as pharmaceuticals and to pharmaceutical compositions containing such macrocycles.

More particularly the present invention provides in a first aspect compounds of formula I



(I)

wherein

R^1, R^2 and R^3 independently are hydrogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;

R^4 is hydrogen, halogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;

R^5 is hydrogen or halogen;

R^6 is hydrogen or C_1-C_4alkyl; and

X is $C=CH_2$ or CHR^6 wherein R^6 is C_1-C_4alkyl which is unsubstituted or substituted by -S-C_1-C_4alkyl.

It will be understood that the above defined compounds may bear substituents within their structure, e.g. may bear appropriate amino moiety substituents.

Alkyl groups and moieties in the compounds of formula I may be branched or straight chain. Alkyl groups are suitably straight chain.

In formula I the following significances are preferred independently, collectively or in any combination or sub-combination:

(a) R^1 is hydrogen or unsubstituted C_1-C_4alkyl, e.g. methyl;

(b) R^2 is hydrogen or C_1-C_4alkyl which is unsubstituted or substituted by OH, e.g. methyl or hydroxymethyl;

(c) R^3 is hydrogen or C_1-C_4alkoxy, e.g. methoxy;

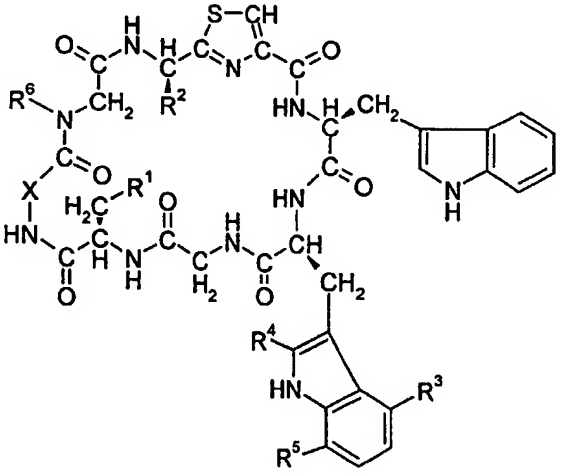
(d) R^4 is hydrogen or unsubstituted C_1-C_4alkyl, e.g. methyl;

(e) R^5 is hydrogen or bromo;

(f) R^6 is hydrogen or methyl; and

(g) X is $C=CH_2$ or CHR^6 wherein R^6 is methyl which is unsubstituted or substituted by -S-ethyl.

More particularly the present invention provides in a further aspect a compound of formula Ia



(Ia)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and X have the above meanings including the preferences.

Examples for compounds of formula Ia include

compound	R^1	R^2	R^3	R^4	R^5	R^6	X
A1	H	CH_3	OCH_3	H	H	CH_3	$C=CH_2$
A2	CH_3	CH_3	OCH_3	H	H	CH_3	$C=CH_2$
A3	H	CH_3	H	H	H	CH_3	$C=CH_2$
A4	H	CH_2OH	OCH_3	H	H	CH_3	$C=CH_2$
A5	CH_3	CH_2OH	OCH_3	H	H	CH_3	$C=CH_2$
A6	H	H	OCH_3	H	H	CH_3	$C=CH_2$

A7	H	CH_3	OCH_3	CH_3	H	CH_3	$C=CH_2$
A8	CH_3	CH_3	OCH_3	CH_3	H	CH_3	$C=CH_2$
A9	H	CH_3	OCH_3	H	H	CH_3	$CHCH_3$
A10	H	CH_3	OCH_3	H	H	CH_3	$CHCH_2SCH_2CH_3$
A11	H	CH_3	OCH_3	H	Br	CH_3	$C=CH_2$
A12	H	CH_3	OCH_3	Br	H	CH_3	$C=CH_2$
A13	CH_3	CH_3	H	H	H	CH_3	$C=CH_2$
A14	CH_3	CH_3	OCH_3	H	H	H	$C=CH_2$

Compounds of formula I may be prepared synthetically by methods known to the skilled person in the art of peptide chemistry, e.g. by a process comprising ring formation of a suitable oligopeptide, or, alternatively, may be isolated from the culture broth of a suitable microorganism. Preparation may include an additional modification step, e.g. hydration of an exocyclic double bond, an addition reaction step or a halogenation step. A suitable microorganism may be identified by culturing a variety of different microorganisms, e.g. selected from the group of myxobacteria and screening the resulting culture broths for the presence of a compound of formula I, in particular for a compound of formula I wherein R^1, R^2, R^3 and R^4 independently are hydrogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy; R^5 is hydrogen; and X is $C=CH_2$. Isolation may follow methods commonly known to the skilled person. A suitable approach may, e.g., comprise culturing the microorganisms in the presence of a solid phase, e.g. a resin, e.g. amberlite, adsorbing the compound of formula I, and, having stopped culturing, eluting the compound of formula I from the solid phase.

A very suitable microorganism for the purposes of the present invention is a strain of the species *Cystobacter minor* (in the present literature, the organism is normally labelled *Cystobacter minus*, which is, however, grammatically not correct) of the order *Myxococcales*, family *Cystobacteriaceae*. This species may be found in soil and rotting plant material worldwide and may be isolated by standard methods.
Cystobacter minor strain Cbm 20, was deposited according to the Budapest Treaty with the DSM (Deutsche Sammlung von Mikroorganismen) in Braunschweig, Germany, on September 4, 2000 by the Gesellschaft für Biotechnologische Forschung under the identification number Cb m20.

The characteristics of the strain correspond to those of the species description as outlined, e.g. in Bergey's Manual of Systematic Bacteriology, volume 3, p.2150, Baltimore: Williams & Wilkins (1989). Vegetative cells are long, slender, needle-shaped, 0.6 to 0.8 / 5 to 10 µm. Myxospores are rod-shaped, short to very short, well rounded, in phase contrast optically refractile, 1.0 to 1.3 / 1.2 to 1.5 µm in size. Fruiting bodies consist of yellow-brown to red brown spherical or somewhat elongated sporangioles, 40 to 60 µm in size, which are arranged in short chains or dense clusters up to 0.5 mm^2 in size. In culture, the fruiting bodies tend to degenerate into undifferentiated knots and narrow ridges, 20 to 30 / 40 to 80 µm in size and often densely packed and fused. The colonies are spreading swarms with long, delicated, radiating veins, often intensely yellow-brown. *Cystobacter minor*, strain Cbm 20 does not degrade chitin. The strain is resistant to ampicillin, sodium salt (50 mg/l) and kanamycin sulfate (250 mg/l).

Suitable culture media include solid and liquid media, e.g. yeast agar, e.g. yeast agar with cells of baker's yeast, which are vigorously decomposed, media containing an enzymatically digested peptone, e.g. Difco Casitone (0.3 %), VY/2-agar (yeast agar, DSM medium 9), CY-agar (DSM medium 67). Cultures may be incubated for, e.g. 2 to 6 days at a temperature of, e.g. 30°C or room temperature, under aerobic conditions. They may be stored frozen, e.g. in a solution of casitone (e.g. 1 %) at –80°C or in liquid nitrogen. If fruiting bodies or at least myxospores are present cultures may be lyophilysed.

Cystobacter minor, strain Cbm 20, and any strain derived therefrom, in particular any strain derived therefrom according to Rule 28 (3) second paragraph European Patent Convention, is also an object of the present invention.

Example 1: Preparation of compounds of formula Ia wherein X is $C=CH_2$ and R^5 is hydrogen

(a) A 350 l bioreactor is charged with 300 l of culture medium containing 0.5 % Probion (single cell protein, Hoechst), 0.1 % calcium chloride, 0.1 % magnesium chloride, 0.1 % yeast extract, 0.5 % starch, 0.2 % glucose, 30 mg of vitamin B_{12}, 3 l of amberlite XAD 16 (Rohm + Haas), and 10 g of 1-docosanol (n-$C_{22}H_{46}OH$) as antifoam agent. After sterilization the pH is adjusted to pH 7.2 with potassium hydroxide, and after inoculation with 2 l of shaking culture of *Cystobacter minor*, strain Cbm 20 kept at pH 7.6 by titration with acetic acid. As antifoam a mixture of 1-dodecanol and methanol 8:2 is added throughout cultivation

at a rate of 0.6 ml/h. The temperature is adjusted to 30°C, aeration is 30 l/min, the impeller is set initially to 150 rpm later adjusted to give an oxygen saturation of 30 %. After 5 days the adsorber resin is harvested by filtration through a 0.5 m^2 process filter (210 µm).

(b) The resin is washed with water to remove adherent cells and is then extracted with 20 l of methanol. The methanol is evaporated and the residual water phase extracted three times with each of 3 l of ethyl acetate. The organic layer is evaporated in vacuo to give a crude extract of 70 g.

This is dissolved in 1 l of methanol and extracted twice with each 0.8 l of n-heptane. The methanol phase is evaporated in vacuo to give a refined extract of 55 g.

Further enrichment is achieved by chromatography on Sephadex LH 20 (column 80 x 20 cm, eluent methanol, 35 ml/min, detection at 254 nm). Based on UV adsorption and tlc monitoring two fractions are taken, a first fraction at 450 - 555 min and a second fraction at 555 - 600 min.

The first fraction is separated on a Merck Präpbar 100 chromatograph (reversed phase column YMC ODS AQ, 120 Å, 15 µm, column dimensions 40 x 10 cm, solvent system methanol/0.05 M Ammonium-acetate buffer 65:35, flow 200 ml/min, detection 227 nm). The first fraction collected (75 - 90 min) yields compound **A1** (R^1 and R^4 are hydrogen, R^2 is methyl and R^3 is methoxy), the second fraction (95 - 120 min) yields compound **A2** (R^1 and R^2 are methyl, R^3 is methoxy and R^4 is hydrogen).

Purification and partial separation of the individual components of the second fraction from the Sephadex column is achieved by RP-18 chromatography (ODS AQ 120 A column from Kronlab, 16 µm, column dimensions 48 x 3 cm, solvent system methanol/0.05 M ammonium acetate buffer 6:4, flow 18 ml/min, detection 227 nm). A first and a second fraction are taken after 80 - 110 min and 110 - 160 min.

Preparative HPLC (Nucleosil C18, Machery Nagel, 7 µm, column 250 x 20 mm, solvent system acetonitrile/water 35:65, flow 8 ml/min, detection 227) nm of the first fraction gives compound **A3** (R^1, R^3 and R^4 are hydrogen, R^2 is methyl). Similarly the second fraction gives compounds **A4** (R^1 and R^4 are hydrogen, R^2 is hydroxymethyl and R^3 is methoxy), **A5** (R^1 is methyl, R^2 is hydroxymethyl, R^3 is methoxy and R^4 is hydrogen) and **A6** (R^1, R^2 and R^4 are hydrogen, R^3 is methoxy).

In analogy to the above procedure compounds **A7** (R^1 is hydrogen, R^2 and R^4 are methyl and R^3 is methoxy) and **A8** (R^1, R^2 and R^4 are methyl and R^3 is methoxy) may be prepared.

The above compounds are found to exhibit the following HPLC retention data [min] [methanol/water 65 :35 + 0.5 % acetic acid/Nucleosil (Macherey Nagel) 125/2 120-5-C18/0.3 ml/min/detection: diode array]:

compound	[min]	compound	[min]	compound	[min]
A1	5.0	A4	3.4	A7	6.3
A2	5.9	A5	3.8	A8	8.2
A3	3.0	A6	3.9		

Example 2: Preparation of compound A9, a compound of formula Ia wherein R^1, R^4 and R^5 are hydrogen, R^2 is methyl, R^3 is methoxy, and X is CHR^6 wherein R^6 is methyl

30 mg (36 µmol) of compound **A1** are dissolved in 4 ml of methanol and palladiumoxide is added (1 spatula tip). The mixture is stirred for six days under an atmosphere of hydrogen, adding fresh catalyst (1 spatula tip) each day. After six days the palladiumoxide is filtered off and the methanol is removed by using a rotary evaporator. The residue is suspended in 500 µl methanol and subjected to preparative HPLC (RP-18, methanol / water = 70 / 30).
The product containing fractions are combined, concentrated and extracted four times with ethyl acetate. The ethyl acetate containing phase is dried over sodium sulfate and the solvent is removed using a rotary evaporator. The residue is dried in high vacuum yielding compound **A9**.

^{1}H-NMR (300 MHz, d_6-Aceton): Ala^2: δ_H= 1.55 (d, $J_{3,2}$ = 7.0 Hz, 3-H_3), 4.63 (m, 2-H)

Example 3: Preparation of compound A10, a compound of formula Ia wherein R^1, R^4 and R^5 are hydrogen, R^2 is methyl, R^3 is methoxy, and X is CHR^6 wherein R^6 is $-CH_2SCH_2CH_3$

15 mg (18 µmol) of compound **A1** are dissolved in 100 µl of methanol and 300 µl (41 mmol) of ethanethiol are added. Potassium hydride (1 spatula tip) is added and the solution is stirred at 60°C. After 2 h a further 100 µl ethanethiol and a spatula tip potassium hydride are added followed by stirring for another 2 h.
The mixture is dried in vacuo, the residue is dissolved in dichloromethane and washed with brine. The organic phase is dried with sodium sulfate and the solvent is evaporated.
According to HPLC/MS analysis (RP-18, methanol / water = 70 / 30) two isomers are obtained (relative ratios): R_t = 2.68 Isomer 1 (46%); R_t = 3.37 Isomer 2 (38%)

The residue is dissolved in 200 µl methanol and separated by preparative HPLC (RP-18, methanol / water = 70 / 30).

Isomer 1: ^{1}H-NMR (300 MHz, d_6-Aceton): δ_H = 1.29 (m, 5-H_3), 2.72 (q, 4-H_2), 4.73 (m, 2-H)

Isomer 2: ^{1}H-NMR (300 MHz, d_6-Aceton): δ_H = 1.18 (m, 5-H_3), 2.56 (q, 4-H_2), 4.86 (m, 2-H)

Example 4: Preparation of compound A11, a compound of formula Ia wherein R^1 and R^4 are hydrogen, R^2 is methyl, R^3 is methoxy, R^5 is bromine and X is C=CH_2

15 mg (18 µmol) of compound A1 are dissolved in 300 µl dichloromethane and 64 µl (40 µmol) of a solution of bromine in dichloromethane are added. After 10 min of stirring dichloromethane is added and the mixture is washed twice with an aqueous solution of sodium thiosulfate, twice with brine and once with water. The organic phase is dried with sodium sulfate and the solvent is evaporated in vacuo. The residue is dissolved in 200 µl of methanol and separated by preparative HPLC (RP-18; methanol / water = 70 / 30). Product containing fractions are united, concentrated to the water phase and extracted four times with ethyl acetate. The ethyl acetate phase is dried over sodium sulfate and the solvent is removed in vacuo.

^{1}H-NMR (300 MHz, d_6-Aceton): δ_H = 6.83 (d, $J_{9,8}$ = 8.6 Hz , 9-H), 7.40 (d, $J_{8,9}$ = 8.1 Hz, 8-H)

Example 5: Preparation of compound A12, a compound of formula Ia wherein R^1 and R^5 are hydrogen, R^2 is methyl, R^3 is methoxy, R^4 is bromine and X is C=CH_2

38,6 mg (46 µmol) of compound **A1** are dissolved in 200 µl of dichloromethane. 9,2 mg (52 µmol) N-bromosuccinimide (NBS) are dissolved in 420 µl dichloromethane and added at 0°C while stirring.

HPLC (methanol / water = 70/30): R_t = 3.49.

The solvent is evaporated in vacuo and the residue is dissolved in 300 µl of methanol. The products are separated by preparative HPLC (RP-18, methanol / water = 70/ 30).

^{1}H-NMR (d_6-Aceton, 300 MHz): δ_H = 6.89 (dd, $J_{8,7/9}$ = 4.7, 3.9 Hz, 8-H) 7.23 (m, 7-H, 9-H)

Starting from compounds **A11** or **A12** compounds **A7** and **A8** may be prepared by alkylation following procedures known to the skilled artisan.

The compounds of the invention and their pharmaceutically acceptable acid addition salts (hereinafter: Pharmaceutical Compounds) have pharmacological activity and are useful as pharmaceuticals as may be demonstrated in animal test methods, e.g. in accordance with the following test methods:

I. Xenotransplantation of syrian hamster heart into rats

The hamster-to-rat heart xenotransplantation model is a so-called difficult concordant model, in which antibody-mediated rejection (3 to 4 days) is the first event. The antibody synthesis occurs in a T-cell independent fashion; congenitally athymic *mu/mu* rats reject a hamster heart in a similar way as euthymic rats.

A compound of formula I is dissolved in a 1+1 mixture of dimethylsulfoxide and a Concentrate For Infusion [650 mg/ml rhicinus oil, polyethoxylated (= Cremophor EL) plus 94 % ethanol ad 1 ml] (COMPOSITION).

Male Syrian hamsters are anaesthetised and the heart is removed into cold physiological saline (4°C).

Male congenitally athymic (*mu/mu*) rats are anaesthetised. A mid-line incision is made from xiphisternum to pubis. The abdominal contents are retracted to the right, to expose the infrarenal abdominal aorta and inferior vena cava. The vessels are dissected free from the fascia for a length of 0.5 to 1 cm, ligating and dividing any small branches (retroaortic spinal branches are preserved). The vessels are clamped individually, first the abdominal aorta. Arteriotomy and venotomy are performed, and lumens are flushed with heparinised physiological saline. Then, the graft is placed in position and correctly oriented. End-to-side aortic anastomosis is made. Then, an end-to-side anastomosis of donor right pulmonary to recipient inferior vena cava is made. The venous clamps are removed, and then distal and proximal aortic clips. Physiological saline is infused intravenously. The abdomen is flooded with warm saline (37°C) and the wound is closed.

Subsequently, COMPOSITION is administered, e.g. by continuous administration for 14 days, e.g. by osmotic minipump.

The graft is monitored daily by palpation of the abdomen. Rejection is concluded in case of cessation of heart beat.

At weekly intervals markers for the presence of antibody-mediated rejection, including destruction of blood vessels and myocyte parenchyma, extravasation of erythrocytes and infiltration by polymorphonuclear granulocytes, are monitored. Compounds of formula I are

effective in this assay when used at a daily dose of in the range of from 0.1 to 200 mg/kg, e.g. 5 to 50 mg/kg, e.g. 10 mg/kg.

II. Allotransplantation of arteries in mice

A compound of formula I is dissolved in a 1+1 mixture of dimethylsulfoxide and Concentrate For Infusion (COMPOSITION).

A mouse donor carotid artery segment is attached in a loop onto the carotid artery of a histoincompatible recipient mouse.

Subsequently, COMPOSITION is administered, e.g. by continuous administration for 14 days, e.g. by osmotic minipump.

Grafts are harvested 30 days after transplantation and markers of vasculopathies including area of the neointima and smooth muscle cell number as described, e.g. in Shi et al., Circ. Res. 75:199-207 (1994) are monitored. Compounds of formula I are effective in this assay when used at a daily dose of in the range of from 0.1 to 200 mg/kg, e.g. 5 to 50 mg/kg, e.g. 10 mg/kg. The inhibition of lymphocyte antigen responses is in the range of about 0.1 to 200 mg/kg, e.g. 5 to 50 mg/kg. The IC_{50} data are in a dosage range of about 0.1 to about 1 μmol. For compound **A2** an IC_{50} of 0.003 μmol has been determined.

Compounds of formula I are, therefore, useful, e.g. as a lymphocyte inhibitor, e.g. a B cell inhibitor.

Compounds of formula I are useful for preventing development of, delaying onset of, delaying progression of, attenuating severity of, suppressing, mitigating or treating graft rejection, e.g. antibody-mediated graft rejection (acute rejection), for preventing graft-versus host diseases, or for treating or preventing transplant vasculopathies (late allograft rejection) in a recipient of e.g. an unmodified or modified organ, tissue or cell allo- or xenograft, e.g. heart, lung, combined heart-lung, trachea, liver, kidney, pancreas, Islet cell, bowel, e.g. small bowel, skin, muscles or limb, bone marrow, oesophagus, cornea or nervous tissue allo- or xenograft.

Compounds of formula I are useful for preventing development of, delaying onset of, delaying progression of, attenuating severity of, suppressing, mitigating or treating autoimmune disorders or diseases. Examples for autoimmune disorders and diseases include antibody or NK cells mediated autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroidis, multiple sclerosis, myasthenia gravis,

type I diabetes and uveitis, and allergies, asthma, psoriasis, sarcoidosis, dermatitis of various etiology, crohn's disease, irritable bowel disease and colitis.

Compounds of formula I are useful for inducing immunological tolerance in a subject in need of such a treatment, e.g. in a cell, tissue or organ transplant recipient. The term "immunological tolerance" refers to a state of unresponsiveness by the immune system of a patient subject to challenge with the antigen to which tolerance has been induced. In the transplant setting, in particular, it refers to the inhibition of the graft recipient's ability to mount an immune response which would otherwise occur in response to the introduction of non-self MHC antigen of the graft into the recipient.

Compounds of formula I are also useful for the treatment of infections, e.g. infections with *Pseudomonas*.

The dosage range of a compound of formula I to be employed for inhibiting lymphocyte antigen responses and the treatment or prevention of graft rejection or treatment of auto-immune disorders or diseases depends upon factors known to the person skilled in the art including host, nature and severity of the condition to be treated and the mode of administration. However, in view of the results in the animal test, satisfactory results are anticipated at daily dosages from about 0.1 to 200 mg/kg, e.g. 5 to 50 mg/kg, preferably administered once daily or continuously via, e.g. minipump. In the case of transplantation administration may start at a day within the range of from, e.g. 14 days prior to transplantation to 14 days post transplantation, in particular at the day of transplantation, and last up to, e.g. 4 weeks, in particular 14 days. Unit dosage forms for administration thus suitably comprise, e.g. from about 0.002 to 100 mg of a compound of formula I together with a pharmaceutically acceptable diluent or carrier therefor.

Compounds of formula I may be administered systemically or topically, by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I may be manufactured in conventional manner, e.g. in the form of a lyophilized powder. The solution for parenteral administration may conveniently be prepared shortly before administration.

The present invention thus provides in further aspects

(1a) a method for inhibiting lymphocyte antigen responses in a subject in need of such a treatment, e.g. in a cell, tissue or organ transplant recipient or in a subject with auto-immune disorders or diseases, which method comprises administering to said recipient or subject an effective amount of a compound of formula I;.

(1b) a method for preventing development of, delaying onset of, delaying progression of, attenuating severity of, suppressing, mitigating or treating graft rejection, e.g. antibody-mediated graft rejection (acute rejection), for preventing graft-versus host diseases, or for treating or preventing transplant vasculopathies (late allograft rejection), in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I;

(1c) a method for inducing immunological tolerance in a subject in need of such a treatment, e.g. in a cell, tissue or organ transplant recipient, which method comprises administering to said subject an effective amount of a compound of formula I.

In a still further aspect the present invention provides

(1d) a method for preventing development of, delaying onset of, delaying progression of, attenuating severity of, suppressing, mitigating or treating autoimmune disorders or diseases in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I.

In a preferred embodiment the invention provides:

(1e) a method of preventing or treating antibody-mediated rejection in an allo- or xeno-cell, -tissue or -organ recipient which method comprises administering to said recipient an effective amount of a compound of formula I.

(1f) a method of transplanting an allo- or xeno-cell, -tissue or -organ in a recipient which method comprises the step of administering to said recipient an effective amount of a compound of formula I.

In further or alternative embodiments the invention provides

(2) a compound of formula I for use as a pharmaceutical;

(2a) a compound of formula I for use as a lymphocyte inhibitor, e.g. a B cell inhibitor, e.g. to prevent or reduce antibody-mediated responses;

(2b) a compound of formula I for use in the prevention or treatment of antibody-mediated rejection of an allo- or xenograft, e.g. an unmodified or modified organ, tissue or cell allo- or xenograft, e.g. heart, lung, combined heart-lung, trachea, liver, kidney, pancreas, Islet cell, bowel, e.g. small bowel, skin, muscles or limb, bone marrow, oesophagus, cornea or nervous tissue allo- or xenograft, in a method as described above;
(2c) a compound of formula I for use in the induction of immunological tolerance;
(2d) a compound of formula I for use as a lymphocyte inhibitor to prevent development of, delay onset of, delay progression of, attenuate severity of, suppress, mitigate or treat autoimmune disorders or diseases;
(2e) a compound of formula I for use in the manufacture of a composition, e.g. for use in a method as described above;
(2f) a compound of formula I for use in the manufacture of a medicament for use as a lymphocyte inhibitor, e.g. in any of the methods as described above;
(3) a pharmaceutical composition comprising a compound of formula I together with one or more pharmaceutically acceptable carriers or diluents therefor;
(3a) a pharmaceutical composition as above for use in any of the methods as described above.

Compounds of formula I may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs in immunomodulating regimens or other anti-inflammatory agents for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders. For example, compound of formula I may be used in combination with cyclosporins, rapamycins or ascomycins, or their immunosuppressive derivatives, e.g. cyclosporin A, cyclosporin G, FK-506, ABT-281, ASM981, rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; FTY720; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD25, CD28, B7, CD40, CD45 or CD58 or their ligands; or other immunomodulatory compounds, e.g. CTLA4Ig, or other adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including Selectin antagonists and VLA-4 antagonists. A preferred composition is with Cyclosporin A, FK506, rapamycin or 40-O-(2-hydroxy)ethyl-rapamycin.

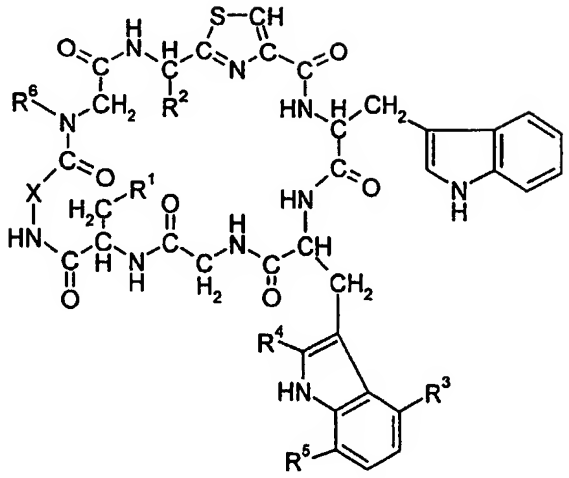
Dosages of the co-administered immunosuppressant or immunomodulatory compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a cyclosporin. Preferably the co-drug employed is an immunosuppressive drug or regimen as used in transplantation, e.g. di- or tri-therapy.

In accordance with the foregoing the present invention provides in a yet further aspect:
(a) a method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a compound of formula I and a second drug substance, said second drug substance being an immunosuppressant or immunomodulatory drug, e.g. as indicated above;
(b) a therapeutic combination, e.g. a kit, for use in any method as defined above, including a pharmaceutical composition comprising a compound of formula I and a pharmaceutical composition comprising an immunosuppressant or immunomodulatory drug. The kit may comprise instructions for its administration.

Investigations so far indicate that compounds of formula I of the invention are well tolerated at dosages required for use in accordance with the present invention.

Claims:

1. A compound of formula I



(I)

wherein

R^1, R^2 and R^3 independently are hydrogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;

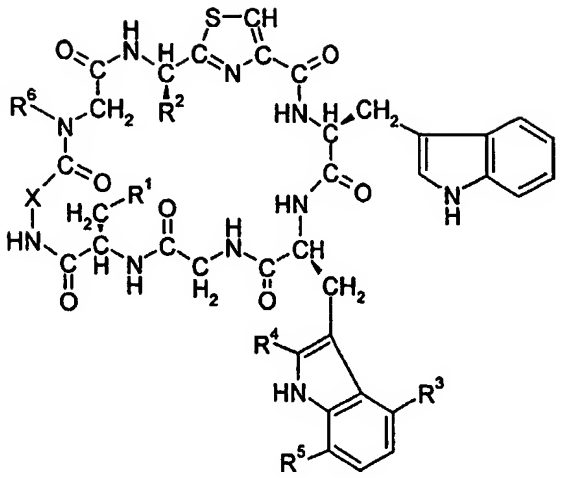
R^4 is hydrogen, halogen, C_1-C_4alkyl which is unsubstituted or substituted by OH, or C_1-C_4alkoxy;

R^5 is hydrogen or halogen;

R^6 is hydrogen or C_1-C_4alkyl; and

X is $C=CH_2$ or CHR^6 wherein R^6 is C_1-C_4alkyl which is unsubstituted or substituted by -S-C_1-C_4alkyl.

2. A compound of formula Ia



(Ia)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and X have the meanings as defined in claim 1.

3. *Cystobacter minor* strain Cbm 20, deposited according to the Budapest Treaty with the DSM (Deutsche Sammlung von Mikroorganismen) in Braunschweig, Germany, on September 4, 2000 by the Gesellschaft für Biotechnologische Forschung under the identification number Cb m20, and any strain derived therefrom.

4. Pharmaceutical composition comprising, or use of, a compound according to claim 1 substantially as hereinbefore described.

5. A compound according to claim 1 for any use substantially as hereinbefore described.

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Application No: GB 0021649.9
Claims searched: 1 at least

Examiner: Peter Davey
Date of search: 24 January 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S):

Int Cl (Ed.7):

Other: Online: CAS ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	Chemical Abstracts 124:219428, see compounds with Registry Nos. 174423-37-1 and 174423-36-0	1 at least
A	Chemical Abstracts 124:197838, see compounds with Registry Nos. 174423-37-1 and 174423-36-0	"

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.